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Gloeocystidiellum kenyense in Azores and Madeira

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Abstract — The aim of this study was to clarify the identity of several collections of *Gloeocystidiellum* from Azores Archipelago and Madeira Island that were initially related to the *Gloeocystidiellum porosum–clavuligerum* group. Three new ITS nrDNA sequences were aligned and analysed phylogenetically with 18 homologous sequences from GenBank. The two main lineages, with very high bootstrap value, that were detected correspond to two well-separated species, *G. kenyense* and G. *clavuligerum*. The sequences obtained from Azores and Madeira specimens clustered in the *G. kenyense* clade. *Gloeocystidiellum kenyense* is described and illustrated from the specimens collected in Macaronesian region, and its affinities with *G. clavuligerum* are discussed.

Key words — *Basidiomycota*, *Gloeocystidiellaceae*, corticioid fungi, oceanic islands, taxonomy

Introduction

In the framework of our studies about corticioid fungi from oceanic islands (Dueñas et al. 2008a, b, Melo et al. 2008, Telleria et al. 2008, 2009a, b, c), we found several collections of a *Gloeocystidiellum* species. These collections from Azores Archipelago and Madeira Island are related, according to Larsson & Hallenberg (2001), to the *Gloeocystidiellum porosum–clavuligerum* group.

Donk (1931) described *Gloeocystidiellum* (*Russulales, Gloeocystidiellaceae*), selecting *G. porosum* (Berk. & M.A. Curtis) Donk as type, to accommodate the corticioid fungi with monomitic hyphal system, gloeocystidia, and smooth or ornamented amyloid spores. Larsson & Larsson (2003) accept *Gloeocystidiellum*

in a strict sense to include a few species with resupinate basidiomes with smooth hymenophores, monomitic hyphal systems, hyphae with clamps, SA+ or SA– gloeocystidia, and ellipsoid, ornamented and amyloid spores.

Several morphological and molecular studies as well as cultural studies and intercompatibility tests have analyzed species delimitation in the *Gloeocystidiellum porosum–clavuligerum* group (Nakasone 1982, Hallenberg 1984, 1988, Boidin et al. 1997, Larsson & Hallenberg 2001, Larsson & Larsson 2002, 2003). ITS2 and LSU nrDNA phylogenetic analyses were congruent with intercompatibility tests and gave higher resolution in differentiating this species complex than could be found in morphologically based analyses. Larsson & Larsson (2002) and Larsson & Hallenberg (2001) found that *G. porosum* and *G. clavuligerum* (Höhn. & Litsch.) Nakasone are phylogenetically widely separated and that *G. clavuligerum* should be divided into two taxa, *G. clavuligerum* and *G. kenyense*.

Our goal was to clarify the identity of *Gloeocystidiellum* specimens from Azores and Madeira mentioned above. ITS nrDNA sequences from three collections were compared with homologous sequences from GenBank published in Larsson & Hallenberg (2001) and Larsson & Larsson (2003). As mentioned in Seifert (2009), the ITS region has became the default marker for species identification in fungi. Morphological characters were compared with the taxa described in Larsson & Larsson (2002).

Materials & methods

Morphological studies

Colors of basidioma are according to ISCC_NBS Centroid Color Charts. Dried specimens were used for light microscope study. Measurements and drawing were made from microscopic sections mounted in 3% KOH solution or Melzer's reagent and examined at up to 1250× with an Olympus BX51 microscope provided with a drawing tube. Lengths and widths of 30 spores, 10 basidia, and 10 cystidia were measured from each sample. Voucher specimens have been deposited in TFC, MA, LISU, O.

DNA extractions and sequencing

Genomic DNA was isolated from herbarium specimens using an EZNA Fungal DNA MiniPrep kit (Omega Biotek, Doraville, Georgia, USA) or a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following instructions of the manufacturers. Lysis buffer incubation was done overnight at 55°C. The primer pair ITS1F and ITS4 was used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flanking parts of the SSU and LSU genes (White et al. 1990, Gardes & Bruns 1993). Amplifications were done using illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK) as described in Winka et al. (1998) following thermal cycling conditions in Martín & Winka (2000). Negative controls lacking fungal DNA were run for each experiment to check for contamination of reagents. Results of amplifications were assayed from 5 µl aliquots

by gel electrophoresis of 2% Pronadisa D-1 Agarose (Lab. Conda, Spain). When the primer pair ITS1F/ITS4 failed, the ITS regions were amplified in two parts as mentioned in Telleria et al. (2010). Prior to sequencing the amplification products were cleaned using a QIAquick gel PCR purification kit (QIAGEN, Valencia, California, USA). Both strands were sequenced separately using primers mentioned above at Secugen S.L. (Madrid, Spain). When products were faintly visualized on agarose gels (less that 20 ng/µl), cloning was conducted with pGEM®-T Easy Vector System II cloning kit (Promega, Madison, Wisconsin, USA). From each cloning reaction, three clones were selected for sequencing, using vector specific primers T7 and SP6 at Macrogen (Korea). Sequences were edited and assembled using Sequencher™ version 4.2 (Gene Codes Corporations, Ann Arbor, Michigan, USA). Blastn searches with megablast option were used to compare the sequences obtained against the sequences in the National Center of Biotechnology Information (NCBI) nucleotide database. The consensus sequences have been lodged in the EMBL-EBI database.

Sequences were manually aligned using Se-Al v2.0a11 Carbon (Rambaut 2002) for multiple sequences and compared with homologous sequences of *Gloeocystidiellum* spp. retrieved from the EMBL Nucleotide Sequence Databases. The alignment was analyzed using the program PAUP*Version 4.0b10 for Macintosh (Swofford 2003). The maximum parsimony analysis (MP) was inferred using the heuristic search option in PAUP. Gaps were treated as missing data. Branch lengths equal to zero were collapsed to polytomies. Nonparametric bootstrap (BP) support (Felsenstein 1985) for each clade was tested based on 10,000 replicates, using the fast-step option.

Results & discussion

From direct PCR with pair primer ITS1F/ITS4, only collections TFC 15278 (GenBank FR878082), TFC 15309 (GenBank FR878083) and MA-Fungi 80408 (GenBank FR878084) were amplified and sequenced directly.

The new sequences included the last 5 bp of 18S nrDNA, the complete ITS1—5.8S—ITS2 sequence, and the first 46 or 50 bp of the 28S nrDNA.

The blast search of the three new direct sequences showed an e-value of 0.0 and a maximum similarity of 99.0% with *G. clavuligerum* sequences (AF310080, AF310077, AF310079, AF310084, AF310082; AF310083) and *G. purpureum* Sheng H. Wu (AF441338). Those GenBank sequences lacked the ITS1 region and the first part of 5.8S nrDNA, thus restricting the Blast search to the last 369 bp at the 3′ end.

The other *G. clavuligerum* sequences from Larsson & Hallenberg (2001) and Larsson & Larsson (2003) as well as the two outgroup *G. porosum* sequences (AF310101 and AF31009) were included in full length in the alignment, which contained 21 sequences and 388 positions, of which 293 were constant, 13 parsimony-uninformative, and 82 parsimony-informative. The 100 most parsimonious trees were obtained using the heuristic search option (tree length = 114; CI = 0.9211; HI = 0.0789, RI = 0.9726). The bootstrap 50% majority-rule consensus tree is shown in Fig. 1.

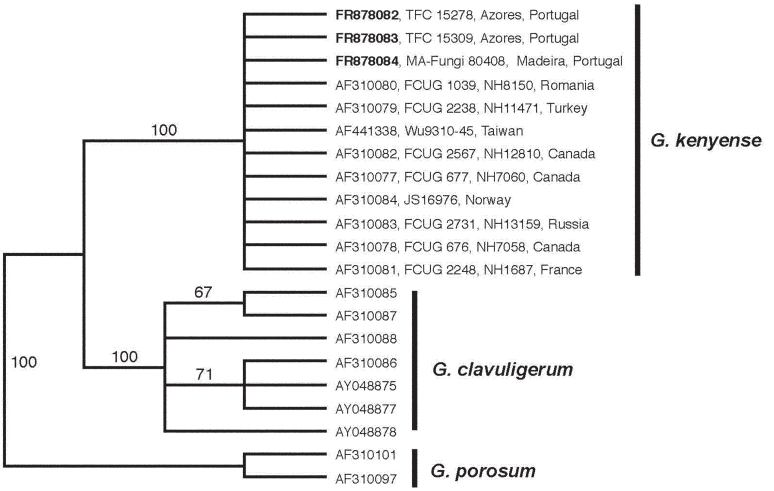

Figure. 1. Strict consensus tree of 100 ITS trees obtained from heuristic search in the parsimony analyses for 21 *Gloeocystidiellum* collections. Bootstrap support values (10,000) above 50% are indicated above branches. The three new sequences generated in this study are labeled with the accession number in bold.

Using *G. porosum* as outgroup, two main clades were detected, both with very high bootstrap values (100%). According to Larsson & Hallenberg (2001) and Larsson & Larsson (2002, 2003), the clades correspond with two well-distinguished species, *G. kenyense* and *G. clavuligerum*. As shown in Fig. 1, the three Azores and Madeira sequences cluster in the *G. kenyense* clade. Comparison of our collections with the *G. kenyense* holotype (L. Ryvarden 8996, herb. O) and protologue description (Hjortstam 1987) revealed no morphological differences.

Gloeocystidiellum kenyense Hjortstam, Mycotaxon 28: 29. 1987 Fig. 2

The Azores and Madeira specimens have resupinate basidiomata that are closely adnate, ceraceous, thick and hard with a smooth cream to ochraceous (89. p. Y–70.l.OY) hymenophore that is cracked when dried and an undifferentiated margin. The hyphal system is monomitic with densely interwoven, richly branched, thin-walled hyphae and clamped at all septa. Gloeocystidia are variable, originate from all levels, 35–85 × 7–16 µm, often with moniliform apical appendices, SA–. Basidia are cylindrical, (13–)21–27 × 4–5 µm, with four sterigmata. Basidiospores are subglobose to ellipsoid, 3.5–5 × 3–3.5 µm [L = 4.1, W = 3, L/W = 1.36], amyloid, ornamented.

Material examined: **PORTUGAL**. **Azores**: Pico Island, S. Roque, Mistério da Prainha, 38°29′42″N 28°16′01″W, 380 m a.s.l., on *Pittosporum undulatum* Vent.

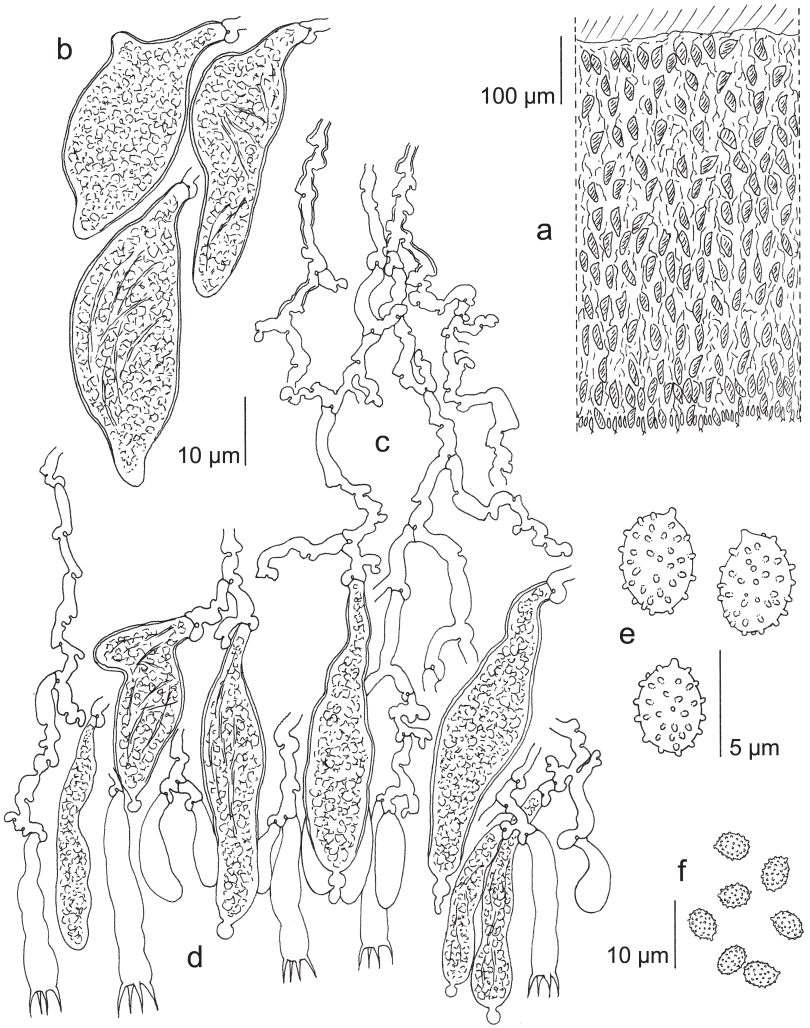

Figure. 2. *Gloeocystidiellum kenyense* (LISU 210747). a: section through basidiome; b: basal gloeocystidia; c: hyphae; d: hymenial layer with basidia and gloeocystidia; e, f: basidiospores.

(*Pittosporaceae*), 25 Febr. 2005, E. Beltrán-Tejera & J.L. Rodríguez-Armas (TFC 15278, GenBank FR878082); S. Roque, Meia Encosta de Santa Luzia, Travessa da Cima, 38°31′13″N 28°22′14″W, 483 m a.s.l., on *Pittosporum undulatum*, 25 Febr. 2005, E. Beltrán-Tejera & J.L. Rodríguez-Armas (TFC 15309, GenBank FR878083). **Madeira**: Porto Moniz, Seixal, Chão da Ribeira, 32°47′56″N 17°06′52″W, 485 m a.s.l., on *Ocotea*

foetens (Aiton) Benth. & Hook.f. (*Lauraceae*), 27 Jun. 2000, M.T. Telleria, 14140Tell. (MA-Fungi 80408, GenBank FR878084); 428 m a.s.l., on unidentified wood, 01 May 1998, I. Melo, 7572IM (LISU 210747). **KENYA**. **Eastern Prov.**: Aberdare Mounts. Kimakia Forest sta., 0°45′S 36°50′W), alt. c. 2200–2400 m a.s.l., 16–18 Jan. 1973, L. Ryvarden 8996 (holotype, O). **TANZANIA**. **Arusha Prov.**: Arusha Nat. Park, Mountain Meru east slope, 1800–2300 m a.s.l., 08 Febr. 1973, L. Ryvarden 10027 (O). **RÉUNION**. Cilaos, 28 March 1990, G. Gilles, Boidin 14289 (O).

According to Larsson & Larsson (2002), *G. kenyense* and *G. clavuligerum* differ in their spore sizes, as well as in gloeocystidial contents, which are SA– and SA+ respectively.

Gloeocystidiellum kenyense is widely distributed and known from Europe, North America, Africa, and Taiwan on decaying angiosperm wood (Larsson & Larsson 2002). In the Azores Archipelago it was found on *Pittosporum undulatum*, an introduced evergreen tree native to SE Australia that today dominates most of the low altitude forests of the Azores archipelago. On Madeira Island the species was found on *Ocotea foetens*, an indigenous species characteristic of the laurisilva, the Macaronesian subtropical mountain moist forest known from Madeira and Canary Islands.

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